The effect of cigarette smoking on serum levels of regulatory cytokines and molecules involved in atherogenesis during systemic treatment of psoriasis – results of a preliminary study

Natalia Zdanowska1, Agnieszka B Owczarczyk-Saczonek1, Joanna Czerwińska1, Jacek J Nowakowski2, Anna Kozera-Żywcyk3, Wojciech T Zdanowski1, Zbigniew Swacha3, Waldemar Placek1

1 Department of Dermatology, Sexually Transmitted Diseases and Clinical Immunology, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Poland
2 Department of Ecology and Environmental Protection, Faculty of Biology and Biotechnology, University of Warmia nad Mazury in Olsztyn, Poland
3 Department of Dermatology, Military Institute of the Health Services in Warsaw, Poland
4 Department of Gynecology and Obstetrics, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Poland

Introduction: Among the diseases associated with psoriasis linked to smoking are primarily cardiovascular diseases (including atherosclerosis) and metabolic syndrome. In addition, cigarette smoking also affects the effectiveness of systemic treatment of psoriasis.

Aim: Assessment of the effect of cigarette smoking on biomarkers of atherosclerosis in patients with psoriasis treated with methotrexate and adalimumab.

Material and methods: The serum levels of vascular cell adhesion molecule 1 (VCAM-1), E-selectin, oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibodies, IL-10, IL-35, TGFß1, were assessed in 34 patients with psoriasis (15 smokers and 19 non-smokers), and 8 healthy, non-smoking volunteers.

Results and discussion: Smoking patients had significantly higher body mass index, lower high density lipoprotein (HDL), higher risk of 10-year fatal cardiovascular disease, higher IL-10 levels and lower IL-35 levels at baseline compared to healthy, non-smoking volunteers. We observed decreases in IL-10, VCAM-1, E-selectin, and oxLDL levels during 12 weeks of methotrexate treatment and, a decrease in IL-35 during adalimumab treatment, based on enzyme-linked immunosorbent assays.

Conclusions: Our results indicate the need for a holistic approach to psoriasis treatment that includes lifestyle modifications like smoking cessation to slow the development of atherosclerosis and increase the possibility of improving skin lesions.
1. INTRODUCTION

Among the causes of mortality that can be prevented, one of the most common is the inhalation of tobacco smoke. Smoking-related cardiovascular diseases (CVDs) are often associated with psoriasis. The results of a recently published meta-analysis indicate that cigarette smoking is a potential risk factor for psoriasis. It influences the onset of the disease and the severity of skin lesions as well as the response to treatment.

Besides the reduction of skin lesions, systemic treatment of psoriasis also has an impact on concomitant diseases. The exact mechanism of methotrexate (MTX) action in psoriasis remains unclear, but it is known to reduce CVD risk and, consequently, mortality through its systemic anti-inflammatory effects, which slow the development of atherosclerosis. Low doses of MTX in patients without accompanying arthritis can decrease the risk of CVD.

The TNF-α inhibitor adalimumab (ADA) weakens the vascular inflammatory response and thus reduces CVD risk. Oberoi et al. (2016) described the inhibitory effect of ADA on atherosclerotic plaque formation by increasing the endothelial adhesion molecules and monocyte adhesion. TNF-α inhibitors, compared to MTX, reduce the risk of cardiovascular events to a greater extent, especially with a longer duration of anti-TNF-α treatment. However, based on the analysis of data from a recently published research study on a large population of patients, no significant differences in the systemic treatment of psoriasis were found with respect to the risk of serious CVD events.

Pathophysiological mechanisms connecting psoriasis with exposure to tobacco smoke include oxidative stress and its associated impact on blood vessels. A recently published meta-analysis describes an increased risk of psoriasis but reduced risk of psoriatic arthritis (PsA) in patients who smoke. Although cigarette smoking is negatively correlated with PsA in psoriatic patients, it is positively correlated with PsA at the population level (the ‘smoking paradox’ of the PsA phenomenon). It is therefore extremely important that patients with psoriasis who are also affected by PsA are strongly encouraged to quit smoking.

Leukocyte migration involving vascular cell adhesion molecule 1 (VCAM-1) and E-selectin during the inflammatory process is the pathophysiological link between psoriasis and atherosclerosis. A high level of VCAM-1 involves a pro-inflammatory immunological imbalance, which is typical of subclinical atherosclerosis. Smoking affects E-selectin levels, indicating its pathophysiological modifications in CVD development.

Interleukin 35 (IL-35) has been described to be atheroprotective and anti-inflammatory in psoriasis. Moreover, it may be a therapeutic target in cigarette smoke-related pulmonary diseases, because its expression provides protective properties against cigarette smoke-induced lung inflammation. Interleukin 10 (IL-10) may be considered as an anti-inflammatory marker; nevertheless, in patients with PsA, it may also contribute to atherosclerosis. Transforming growth factor β1 (TGFβ1) may increase the risk of coronary artery disease (CAD), but its role in psoriasis requires clarification.

Cigarette smoke exposure promotes the formation of oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibodies. High levels of anti-oxLDL antibodies are observed in atherosclerosis associated with smoking. However, obesity is described as a stronger predictor of oxLDL formation than smoking in post-menopausal women. OxLDL antigens initiate the process of plaque formation, but their exact role in atherosclerosis requires elucidation. High plasma oxLDL and smoking lead to instability of atherosclerotic plaque in patients with peripheral artery disease (PAD). Accumulation of oxLDL deposits in the skin may be responsible for skin damage, and the anti-oxLDL levels may be used as a potential marker of early atherosclerosis in patients with psoriasis.

The definitive indicator of severity of skin lesions during psoriasis (psoriasis area and severity index – PASI or body surface area – BSA) that best correlates with CVD risk has not been established. The specific threshold of PASI or BSA from which systemic treatment of psoriasis should be started to prevent increased CVD risk is also unclear.

2. AIM

The purpose of the present study was to determine the influence of smoking and systemic treatment (MTX and ADA) on the level of molecules involved in the pathogenesis of atherosclerosis (VCAM-1, E-selectin, oxLDL, anti-oxLDL) and regulatory cytokines (IL-10, IL-35, TGFβ1) in patients with plaque psoriasis.

3. MATERIAL AND METHODS

A detailed description of the studied group, the methods used, and the statistical analysis employed has been already published. The study group comprised 34 patients (27 men and 7 women) aged 30–73 years with moderate to severe plaque psoriasis. The control group consisted of 8 healthy volunteers aged 30–57 years.

The patient inclusion criteria were age over 18 years and a clinical diagnosis of moderate to severe plaque psoriasis. The exclusion criteria were age below 18 years, mild plaque psoriasis, pregnancy, and breastfeeding. The control group joined the study as volunteers and their selection was random.

In the study group, 17 patients were treated only with oral MTX at a dose of 7.5–20.0 mg per week using folic acid supplementation 24 h after treatment. The remaining 17 patients received ADA subcutaneously with a first dose of 80 mg, followed by 40 mg every 2 weeks. The study period lasted 12 weeks.

Laboratory tests were performed using an enzyme-linked immunosorbent assay (ELISA) with fasting blood samples taken before and after 12 weeks of treatment. The serum concentrations of IL-10, IL-35, and TGFβ1 were determined using a commercial ELISA kit (Biorbyt Ltd, Cambridge, UK) and FineTest (Wuhan Fine Biotech Co Ltd, Wuhan, China). Serum levels of VCAM-1, E-selectin, oxLDL, and anti-oxLDL were measured using commercial ELISA kits (Wuhan Elab Science Co Ltd, Wuhan, China; Immundiagnostik
Table 1. Initial (W₀) characteristics of the study group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MTX n = 17</th>
<th>ADA n = 17</th>
<th>Control n = 8</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0.01c</td>
</tr>
<tr>
<td>no</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m², range (mean ± SD)</td>
<td>23.1–39.8</td>
<td>24.0–42.4</td>
<td>19.1–33.0</td>
<td>0.05c</td>
</tr>
<tr>
<td>SCORE, %, range (mean ± SD)</td>
<td>&lt;1–10</td>
<td>&lt;1–8</td>
<td>&lt;1–1</td>
<td>0.0012c</td>
</tr>
</tbody>
</table>

Comments: Significant differences are marked in bold; a 2 test; b one-way ANOVA model; c post-hoc test: MTX vs. ADA; d post-hoc test: MTX vs. control; e post-hoc test: ADA vs. control; f Kruskal–Wallis test.

Here, we present the results of secondary analysis concerning the impact of cigarette smoking and systemic treatment of psoriasis on previously studied regulatory cytokines and molecules involved in atherogenesis. Among the patients with psoriasis, 15 were current cigarette smokers and 19 were non-smokers. The 8 healthy volunteers that comprised the control group were non-smokers without a history of inflammatory or dermatologic diseases. The number of current cigarette smokers and non-smokers in the MTX group were 10 and 7, respectively. Five of the patients treated with ADA were smokers; 12 were non-smokers.

Table 2. The influencing effect of smoking on psoriasis severity indicators at the beginning (W₀) and at the completion of the observation (W₁₂).

<table>
<thead>
<tr>
<th>Psoriasis severity index</th>
<th>Week</th>
<th>Smokers, mean ± SD</th>
<th>Non-smokers, mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASI, points</td>
<td>W₀</td>
<td>15.3 ± 7.22</td>
<td>17.21 ± 6.35</td>
<td>0.3</td>
</tr>
<tr>
<td>W₁₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA, %</td>
<td>W₀</td>
<td>25.1 ± 12.86</td>
<td>28.14 ± 17.73</td>
<td>0.7</td>
</tr>
<tr>
<td>W₁₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments: Significant differences are marked in bold. a Mann–Whitney U test, smokers vs. non-smokers.

Table 3. Differences in SCORE risk, BMI, and lipid profile between smokers and non-smokers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Smokers n = 15</th>
<th>Ps n = 19</th>
<th>Non-smokers C n = 8</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>50.86 ± 8.7</td>
<td>48.42 ± 15.82</td>
<td>34.62 ± 9.37</td>
<td>1.0b</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
<td></td>
<td></td>
<td>0.0097c</td>
</tr>
<tr>
<td>male</td>
<td>13</td>
<td>14</td>
<td>2</td>
<td>0.039b</td>
</tr>
<tr>
<td>female</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m², mean ± SD</td>
<td>31.68 ± 5.56</td>
<td>28.52 ± 3.88</td>
<td>22.61 ± 5.14</td>
<td>0.18</td>
</tr>
<tr>
<td>SCORE, %, mean ± SD</td>
<td>6.23 ± 7.45</td>
<td>2.86 ± 2.77</td>
<td>0.56 ± 0.17</td>
<td>0.34e</td>
</tr>
<tr>
<td>Cholesterol, mg/dL, mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td>0.0011f</td>
</tr>
<tr>
<td>Total</td>
<td>217.73 ± 39.79</td>
<td>199.47 ± 45.58</td>
<td>210.5 ± 24.62</td>
<td>0.42</td>
</tr>
<tr>
<td>HDL</td>
<td>41.95 ± 8.91</td>
<td>47.94 ± 10.79</td>
<td>65.0 ± 9.28</td>
<td>0.23e</td>
</tr>
<tr>
<td>LDL</td>
<td>142.52 ± 41.65</td>
<td>120.75 ± 36.17</td>
<td>128 ± 28</td>
<td>0.25b</td>
</tr>
<tr>
<td>Triglycerides, mg/dL, mean ± SD</td>
<td>178.53 ± 134.72</td>
<td>141.31 ± 63.64</td>
<td>87.25 ± 28.68</td>
<td>1.0e</td>
</tr>
</tbody>
</table>

Comments: Significant differences are marked in bold; b patients with psoriasis smokers vs. control group W₀; c patients with psoriasis smokers vs. control group W₁₂; d post-hoc test; e analysis of variance.

4. RESULTS

Table 1 contains the characteristics of the subjects at the beginning of the study period (W₀). Patients with psoriasis had a significantly higher BMI compared with that of the control group (P = 0.03 and P = 0.05 for patients assigned to treatment with MTX and ADA, respectively). Cardiovascular risk (as estimated via a SCORE chart) in patients qualified for treatment with MTX was significantly higher than in the control group (P = 0.001).

Table 2 outlines the effect of smoking on psoriasis severity indicators (PASI and BSA) and demonstrates the absence of any impact of cigarette smoking on achieving clinical improve-
ment in skin lesions among the patients included in the study. Smoking had no effect on achieving at least 75% (PASI-75) and at least 90% (PASI-90) reduction in PASI after 12 weeks of treatment ($P = 0.15$ and $P = 0.12$, respectively, Fisher’s exact test).

Table 3 shows the differences between the lipid profile, BMI, and CVD risk (SCORE) among the cigarette smoking and non-smoking patients included in this study. At the baseline, comparing smoking and non-smoking patients with psoriasis, there were no significant differences between the groups. Smoking patients with psoriasis were significantly older than the healthy, non-smoking controls ($P = 0.0097$, Kruskal-Wallis test). BMI ($P = 0.01$, analysis of variance), SCORE ($P = 0.001$, Kruskal-Wallis test), as well as triglycerides ($P = 0.02$, Kruskal-Wallis test) were significantly higher in the smokers, while high-density lipoprotein (HDL) levels were significantly higher in the controls ($P = 0.0002$, analysis of variance). The non-smoking patients with psoriasis had a significantly higher BMI ($P = 0.046$, analysis of variance) and were significantly older ($P = 0.039$, Kruskal-Wallis test) than the healthy, non-smoking volunteers.

Table 4 presents the levels of studied molecules in smokers and non-smokers at the initiation (W0) and completion (W12) of the study. At W0, comparing smoking and non-smoking patients with psoriasis, there were no significant differences in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Smokers</th>
<th>Non-smokers</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxLDL ng/mL, mean ± SD</td>
<td>0.65 ± 0.42</td>
<td>0.58 ± 0.48</td>
<td>0.79 ± 0.54</td>
</tr>
<tr>
<td>anti-oxLDL U/mL, mean ± SD</td>
<td>37138.41 ± 17738.85</td>
<td>33983.58 ± 20909.06</td>
<td>33421.4 ± 22206.4</td>
</tr>
<tr>
<td>IL-10 pg/mL, mean ± SD</td>
<td>841.91 ± 1946.94</td>
<td>294.05 ± 400.43</td>
<td>592.25 ± 1626.39</td>
</tr>
<tr>
<td>IL-35 pg/mL, mean ± SD</td>
<td>22.26 ± 21.70</td>
<td>17.19 ± 12.79</td>
<td>35.65 ± 27.85</td>
</tr>
<tr>
<td>TGFβ1 pg/mL, mean ± SD</td>
<td>11127.89 ± 5862.4</td>
<td>12706.18 ± 3907.11</td>
<td>12537.9 ± 4580.55</td>
</tr>
<tr>
<td>VCAM-1 ng/mL, mean ± SD</td>
<td>0.74 ± 0.46</td>
<td>0.44 ± 0.43</td>
<td>0.74 ± 0.52</td>
</tr>
<tr>
<td>E-selectin, ng/mL, mean ± SD</td>
<td>1 ± 1.37</td>
<td>0.42 ± 0.49</td>
<td>0.73 ± 0.57</td>
</tr>
</tbody>
</table>

Comments: Significant differences are marked in bold; Ps – non-smoking patients with psoriasis; C – non-smoking healthy volunteers; *Kruskal-Wallis test; **patients with psoriasis smokers vs. control group W0; ***patients with psoriasis no-smokers vs. control group W0; †Mann–Whitney U test; ‡smokers vs. non-smokers W12; ††Wilcoxon test; ‡‡smokers W0 vs. W12; ‡‡‡non-smokers W0 vs. W12; †††t-test.
serum levels of the studied molecules. However, when comparing smoking psoriasis patients and healthy, non-smoking volunteers, significantly higher levels of oxLDL, VCAM-1 and E-selectin were found ($P = 0.02$, $P = 0.0003$, $P = 0.0003$, and $P = 0.0007$, respectively, Kruskal-Wallis test). The levels of oxLDL, IL-10, VCAM-1, and E-selectin were also significantly higher ($P = 0.004$, $P = 0.004$, $P = 0.0003$, and $P = 0.001$, respectively, Kruskal-Wallis test), in the non-smoking patients with psoriasis compared to the non-smoking volunteers. At W12, the plasma concentrations of oxLDL, anti-oxLDL, IL-35, and TGFβ1 in patients with psoriasis from both groups did not differ significantly between the cigarette smoking and non-smoking groups. Whereas, the smoking patients exhibited a significant decrease in VCAM-1, E-selectin, and IL-10 ($P = 0.006$, $P = 0.001$, and $P = 0.008$, respectively, Wilcoxon test). Non-smokers, however, showed a significant increase in VCAM-1 ($P = 0.04$, Wilcoxon test) and a decrease in IL-35 ($P = 0.002$, Wilcoxon test).

Figure 1 shows the differences in the levels of the studied molecules in smoking and non-smoking patients, depending on the treatment method used (MTX or ADA) during the 12 weeks of therapy. There was a significant decrease in IL-35 levels in non-smoking patients treated with ADA.
The findings of our research indicate that cigarette smoking affects the levels of adhesion molecules, regulatory cytokines and oxLDL during systemic treatment of plaque psoriasis. Decreases in IL-10, VCAM-1, E-selectin, and oxLDL levels were observed during 12 weeks of treatment with MTX, and, surprisingly, a decrease in IL-35 occurred during treatment with ADA. There was no correlation between smoking and the severity of psoriasis or the effectiveness of the treatment (obtaining PASI-75 and PASI-90).

Patients with psoriasis have an increased risk of cardiovascular mortality. In our study, CVD risk (estimated via SCORE charts) was higher in patients with psoriasis at the beginning of treatment with MTX than in the healthy volunteers. It was also clearly higher in the smokers than in the non-smokers.

The higher the BMI, the greater the risk and severity of psoriasis development. This also entails increased risk of side effects during MTX treatment. During long-term anti-TNFα treatment, an increase in BMI was observed, which may have reduced the effectiveness of treatment.

In our study, at baseline, patients with psoriasis who were treated with MTX and ADA had significantly higher BMI values compared with those of control group participants. Non-smokers had a significantly lower BMI than that of the cigarette smokers.

Anzengruber et al. (2019) did not find a lower response rate – defined as reaching a PASI reduction of at least 75%, PASI ≤ 3, or dermatology life quality index (DLQI) up to 1 – to systemic anti-psoriatic therapies in smokers. Similarly, in our study, the improvement (understood as achieving PASI-75 and PASI-90) observed after 12 weeks of treatment was no less in smokers than in non-smoking patients with psoriasis.

Smoking leads to a decrease in HDL cholesterol, and an increase in triglyceride levels. Higher values of triglycerides and HDL cholesterol have been described among women with psoriasis with a history of smoking. In our study, the HDL concentration was significantly higher in non-smokers than in cigarette smokers.

Patients with psoriasis are characterized by increased plasma levels of VCAM-1 and E-selectin. E-selectin-elevated serum concentrations are also correlated with disease severity. However, decreases are not connected with clinical improvement after treatment. Nevertheless, after 3 months of ADA treatment for psoriasis, the serum concentration of E-selectin decreases. Furthermore, tobacco smoking is associated with a high level of serum E-selectin in patients with migraines. Several studies have described the protective role of HDL against increased E-selectin. In patients with early stages of rheumatoid arthritis, a connection has been observed between low disease activity and increased serum concentrations of VCAM-1 after a 3-month treatment with methotrexate. The increase in VCAM-1 leads to a pro-inflammatory imbalance, indicating the presence of subclinical atherosclerosis in non-smoking patients in the initial stage of metabolic syndrome. At the beginning of our study, there were no significant differences in the plasma concentrations of VCAM-1 and E-selectin between smokers and non-smokers. After 12 weeks, a decrease in E-selectin and VCAM-1 concentrations was observed in MTX-treated smokers.

Serum oxLDL has been shown to be significantly higher in patients with psoriasis than in healthy subjects. In addition, oxLDL is significantly higher in smokers than non-smokers. Elevated serum anti-oxLDL antibodies can be correlated with atherosclerosis. In our study, oxLDL levels decreased significantly in smokers during MTX treatment.

TGFβ1 levels are often low in smokers, which may increase the risk of CAD. It was found that increased serum levels of IL-10 in patients with psoriasis was present at low levels in smokers. Plasma concentrations of IL-10 and IL-35 are negatively correlated with the smoking status of the individual. Plasma concentrations of IL-35 in cigarette smokers have not been previously investigated. Compared with healthy individuals, IL-35 serum levels are low in patients with psoriasis and increase after treatment. Solberg et al. found that increased serum levels of IL-10 in patients with psoriasis is correlated with the effect of biological treatment (anti TNFα, anti-IL-12/23, anti IL17A, and TNF-receptor blocker).

In our study, IL-35 serum levels at baseline in smoking psoriasis patients (both MTX- and ADA-treated groups) was significantly lower than those of non-smokers, whereas IL-10 levels were significantly higher in the smokers. At the end of the study, the plasma concentrations of all studied molecules in patients with psoriasis patients from both MTX- and ADA-treated groups did not differ significantly between the smokers and non-smokers. During the 12-week study period, IL-10 levels dropped significantly in patients treated with MTX, but unexpectedly, IL-35 levels dropped significantly in non-smoking patients treated with ADA. This decrease can be explained by the proven inhibition of Th1 lymphocyte proliferation by IL-35 and its negative correlation with PASI. Therefore, during successful treatment, an increase in IL-35 was expected.

The inconsistent results of patients included in this study may be due to limitations of the study, such as differences in the BMI of the smokers and non-smokers, the short
observation time, and the small sample number of patients. To draw wider conclusions, we plan to study a larger group of people over a considerable period of time.

6. CONCLUSIONS

The implications of our research are complementary to the present understanding of the consequences of systemic MTX and anti-TNFα antibody treatment of plaque psoriasis in cigarette smoking and non-smoking patients in terms of the regulatory cytokines, cell adhesion molecules, oxLDL, and anti-oxLDL. Understanding the influence of smoking, not only on the severity of psoriasis, but also on molecules that may be markers of early subclinical atherosclerosis, may facilitate an appropriate choice of treatment for these patients.

Conflict of interests
Zbigniew Swacha cooperates with Medac. Other authors declare no conflict of interests.

Both funders and Medac had no influence on the research project, collection, analysis and interpretation of the results obtained, manuscript writing and the decision to publish the obtained conclusions.

Funding
This study was supported by the Ministry of Science and Higher Education, Poland (grant No. 61.610. 001-300).

Data sharing statement
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethics
The study was approved by the Bioethics Committee of the University of Warmia and Mazury in Olsztyn, Poland (Resolution 16/2019). Informed consent was obtained from each patient enrolled in the study.

References


